NTP
National Toxicology Program

NTP Research Concept - Deoxynivalenol

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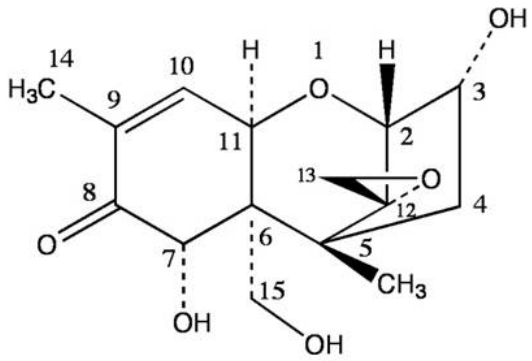
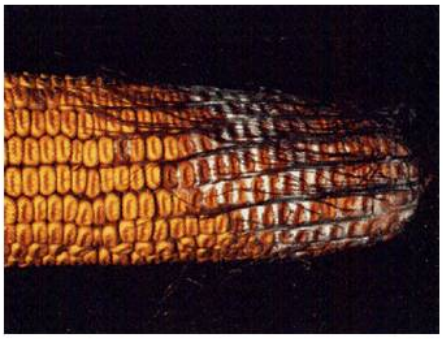
Nomination Background and Rationale

- Deoxynivalenol (DON), is a trichothecene mycotoxin
  - Produced by certain Fusarium species
  - Frequently occurs on corn, wheat, barley, rice, and other grains
- Nominated by the NIEHS for chronic toxicity, carcinogenicity and reproductive toxicity studies based on:
  - Possible contamination of human foods
  - Demonstrated toxicological activity
  - Lack of definitive long-term studies



DON Exposure

- Human and animal exposure through the consumption of contaminated grains
  - Highly stable during processing
  - Does not degrade at high temperatures
- Acute toxicity manifested as gastrointestinal distress
- Elevated DON levels associated with periods of increased rainfall
- May be occupational exposure in farmers engaged in threshing, where high concentrations may occur in grain dusts



NTP Studies - DON Exposure Assessment

- Collaboration with Dr. Jane Hoppin, NIEHS and Dr. Donald Beezhold, NIOSH
- Evaluation of allergic sensitization in a subset of farmers from the NIEHS Agricultural Health Study
- Screening ~700 serum samples for total IgE, mold-specific IgEs and DON levels
- Examining associations with respiratory outcomes, allergic disease and neurologic function

DON - Epidemiology and Genotoxicity Studies

- Few epidemiology studies
  - Elevated DON levels found in several areas in China with high esophageal cancer rates
  - Most studies correlate grain intake with urinary levels of DON, rather than with specific disease risk
- Genotoxicity
  - Published data suggests that gene mutations are NOT induced in bacterial or *in vitro* assays in mammalian cells
  - Positive for induction of DNA damage or chromosomal aberrations in *in vitro* assays using mammalian cells
    - Experimental details are generally lacking
    - One study reported a requirement for S9 activation

DON Toxicity Studies - Short term and subchronic in rodents

- Short-term and subchronic exposure to DON decreased body weight, weight gain and feed consumption in rats and mice
- Rats
  - Most studies show decreases in body weight gain in one or both sexes
  - Some evidence of histological alterations in gastrointestinal tract and spleen but not in all studies
    - Arnold et al., 1986
    - Morrissey and Vesonder, 1985

DON Toxicity Studies - Short term and subchronic in rodents

- Mice
  - Effects are strain and dose-dependent with some deaths at doses ≥ 2.5 mg/kg
  - Lesions in the spleen, thymus, lymph nodes, and gastrointestinal tract and changes in bone marrow and hematological parameters at doses ≥ 2.5 mg/kg
  - Decreased food intake and weight gain, changes in organ weights even at very low doses
    - Robbana-Barnat et al., 1987
    - Arnold et al., 1986
    - Pestka et al., 1986

DON - Effect on Male Reproductive Tissues

- Rats
  - 28 day gastric intubation with a NOEL of 1.0 mg/kg (Sprando et al., 2005)
  - Dose related decrease in spermatid counts and numbers in the testes and epididymis
  - Decreased epididymal and seminal vesicle weights
  - Increased germ cell degeneration, sperm retention and abnormal morphology
- Mice
  - 90 day feeding study at 10 ppm in mice (Sprando et a., 1999)
  - Decreased epididymal weight in the absence of changes in sperm count
  - No effects on testes or testicular weights

DON - Reproductive Toxicity Studies

- Rats
  - SD rats fed up to 1 mg/kg DON for 6 weeks (Khera et al., 1984)
    - No effects on reproduction, but reduced body weight in the dams
  - SD rats fed 2 mg/kg DON (Morrissey and Vesonder, 1985)
    - Lower pregnancy rates in treated females
    - No effects on sex ratio, pup survival, number of pups or litter weights
- Mice
  - Swiss Webster mice fed up to 2 mg/kg DON for 30 days (Khera et al., 1984)
    - Reduced numbers of live pups, postnatal survivors and postnatal body weight
    - Cross-fostering studies suggest that both prenatal and perinatal exposures adversely affected pup survival and body weight

DON - Developmental Toxicity Studies

- Rats
  - No developmental effects in F344 rats exposed to dosed feed containing up to 5 mg/kg DON (Morrissey, 1984)
    - Minimal maternal toxicity, though some decrease in maternal body weight
  - Skeletal anomalies and increased fetal resorptions in SD rats treated via oral gavage at DON doses > 2.5 mg/kg (Collins et al., 2006)
    - At 2.5 and 5 mg/kg bw/day, average fetal body weight, crown-rump length, and ossification of fetal vertebrae were significantly decreased.
    - Significant increases in the average number of early and late deaths per litter, the incidence of runts, and the incidence of misaligned and fused sternebrae
    - NOAEL for fetal toxicity was 1 mg/kg and that for maternal toxicity was 0.5 mg/kg
- Mice
  - Visceral and skeletal malformations in fetuses from Swiss Webster dams fed > 1 mg/kg (Khera et al., 1982)
  - Skeletal anomalies and increased fetal resorptions in NMRI mice following i.p. injection of up to 10 mg/kg DON

The interpretation of these studies is complicated by the difficulties in separating the pharmacologic effects on the developing organism from the gastrointestinal effects that induce maternal toxicity

DON - Cancer and Immunology Studies

- Carcinogenicity
  - Joint FAO/WHO Expert Committee on Food Additives and the European Commission Scientific Committee on Food identified the lack of a cancer study in a second species as a significant data gap
  - 2 year carcinogenicity study via dosed feed in B6C3F1 mice (Health Canada)
    - Significant body weight reduction in male and female mice fed 5 and 10 ppm DON
    - Food consumption decreased in male mice at 5 and 10 ppm DON
    - DON produced a dose-related decrease in liver preneoplastic and neoplastic lesions in males only which was attributed to reduced caloric intake
- Immunotoxicity
  - DON modulates immune function and host resistance in mice
  - Considerable work has been done on mechanism of action and comparison with other mycotoxins

DON - Toxicokinetics

- Rapid absorption in B6C3F1 mice, biphasic elimination
- Limited toxicokinetics data for rats
- Information on the bioavailabilty, mass balance, and metabolism not available for the routes reported in the published toxicokinetics studies
- Based on the available data, there appear to be differences in toxicokinetics based on route of exposure and species

Proposed Research Program - Key Issues

- Although there is considerable data on the toxicity of DON, many of the studies, in particular several of the studies of effects on reproduction and development are difficult to interpret because of significant overt toxicity to the dam and inadequate experimental design
- Studies on reproductive and developmental toxicity with appropriate routes of exposure and doses, including an assessment of effects on fertility in the male should be conducted
- A chronic study in a second species (rat) is a data gap that needs to be addressed
- Since humans may be exposed to a variety of tricothecene mycotoxins in the diet, the establishment of toxic equivalency factors (TEFs) for may be important in assessing cumulative risk

Proposed Research Program (continued)

- The overall goal of these studies is to characterize the reproductive, chronic toxicity and carcinogenicity of DON following oral exposure in rats
- The following tiered approach with defined specific aims will address this goal:
- Tier One
  - Specific Aim 1 - Toxicokinetics studies in Harlan Sprague Dawley (SD) rats and B6C3F1 mice
    - These studies should be done in conjunction with or prior to the prechronic toxicity studies
    - These studies will provide information on the bioavailability and tissue distribution of DON
    - Mouse studies will address issue of species differences in sensitivity

Proposed Research Program (continued)

- Tier 1 (continued)
  - Specific Aim 2 - Conduct prechronic dosed feed toxicity studies in the SD rat
    - These studies will provide critical information for dose setting in the RACB and chronic toxicity studies
    - These studies will provide data needed to set TEF values for DON
  - Specific Aim 3 - Evaluate the genotoxicity of DON
    - A stand alone genotoxicity study will be conducted in rats and mice to determine if exposure to DON induces chromosomal damage in vivo

Proposed Research Program (continued)

- Tier Two
  - Specific Aim 4 - Conduct a guideline reproductive toxicity study to examine the effects of DON on fertility and fecundity in SD rats
  - Specific Aim 5 - Conduct a 2 year dosed feed chronic toxicity and carcinogenicity study in SD rats

Significance and Expected Outcomes

- The proposed studies will address data needs for DON that have been articulated by several groups, including the need for a cancer study in a second species and studies of the effects on reproduction in males
- These definitive studies will provide necessary information for evaluating the cumulative risk from exposure to DON and other tricothecene mycotoxins
- Additional toxicology studies may identify more sensitive endpoints relative to human exposures